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(54) Title: USE OF 2-AMINO-4-(4-FLUORONAPHTH-1-YL)-6-ISOPROPYLPYRIMIDINE IN THE TREATMENT OF GI DISORDERS

(57) Abstract: The present invention relates to the use of 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine, and salts or N-oxides thereof, in the treatment of disorders of the gastro-intestinal tract, in particular for the treatment of irritable bowel syndrome (IBS).

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USE OF 2-AMINO-4-(4-FLUORONAPHTH-1-YL)-6-ISOPROPYLPYRIMIDINE
IN THE TREATMENT OF GI DISORDERS

The present invention relates to the treatment of disorders of the gastro-intestinal (GI) tract, in particular the treatment
5 of irritable bowel syndrome (IBS).

Background to the invention.

Serotonin, also referred to as 5-hydroxytryptamine (5-HT), is a neurotransmitter with mixed and complex pharmacological characteristics. 5-HT acts via a number of discrete 5-HT
10 receptors. Currently, fourteen subtypes of serotonin receptor are recognized and delineated into seven families, 5-HT₁ to 5-HT₇. Within the 5-HT₂ family, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} subtypes are known to exist. The nomenclature and classification of 5-HT receptors has been reviewed by Martin and Humphrey,
15 Neuropharm., 33, 261-273 (1994) and Hoyer et al., Pharm. Rev., 46, 157-203 (1994).

5-HT_{2B} receptors, initially termed 5-HT_{2F} or serotonin-like receptor, were first characterized in rat isolated stomach fundus (Clineschmidt et al., J. Pharmacol. Exp. Ther., 235,
20 696-708 (1985) and Cohen and Wittenauer, J. Cardiovasc. Pharmacol., 10, 176-181 (1987)).

WO97/44326 describes aryl pyrimidine derivatives and their use as selective 5-HT_{2B} antagonists. Of the numerous compounds described, 2-amino-4-(4-fluoronaphth-1-yl)-6-
25 isopropylpyrimidine is mentioned. Bonhaus et al, Br. J. Pharmacol. 1999, 127, 1075-1082, report that this compound (RS127445) is a selective, high affinity, 5-HT_{2B} receptor antagonist.

IBS is a common disorder, characterised by chronic and

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recurrent gastrointestinal symptoms such as altered bowel function, pain, flatulence, bloating and constipation and/or diarrhoea, but for which there is no apparent biochemical or structural cause. IBS is not associated with any general
5 deterioration in health. While the cause of IBS is unknown, the condition is often associated with stress or anxiety.

Current approaches to the treatment of IBS include the antagonism of 5-HT at 5-HT₃ receptors, and either mimicking or blocking the effects of 5-HT at 5-HT₄ receptors in the
10 gastrointestinal tract. Development of 5-HT₄ and 5-HT₃ antagonists was based upon observations in animal studies, where both types of receptors are present on myenteric neurones, and mediate the enhancement of the release of excitatory neurotransmitters which cause contraction of
15 gastrointestinal smooth muscle.

Disclosure of the invention.

The present invention is based upon the finding that whereas current approaches to the treatment of IBS include the antagonism of 5-HT at 5-HT₃ receptors, and either mimicking or
20 blocking the effects of 5-HT at 5-HT₄ receptors in the gastrointestinal tract, we have found that in the human GI tract 5-HT_{2B} receptors are present on myenteric neurones, and smooth muscle and that these receptors mediate the enhancement of the effects of neuronal stimulation, causing a potentiation
25 of the contraction of GI smooth muscle that results from stimulation of the myenteric neurones. This potentiation of the neuronally-mediated contractile response may be inhibited or eliminated by the application of antagonists at 5-HT_{2B} receptors. The localisation of functional 5-HT_{2B} receptors on
30 human myenteric neurones has not previously been demonstrated in humans or indeed in any other species.

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The present invention provides a method for the treatment of a disorder of the GI tract, which method comprises administering to a subject in need of treatment, an effective amount of 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine, or a salt or N-oxide thereof.

The invention further provides the use of 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine, or a salt or N-oxide thereof, in the manufacture of a medicament for the treatment of a disorder of the GI tract.

10 The compound may be in the form of a composition comprising said compound together with a pharmaceutically acceptable diluent or carrier.

The disorder of the GI tract is in particular IBS.

Detailed description of the invention.

15 IBS.

The diagnosis of IBS is difficult due to the plethora of symptoms and the lack of reliable diagnostic tests. Diagnosis is often made on the basis of careful questioning of the patient but in patients with diarrhoea, other conditions such as inflammatory bowel disease (IBD), infective gastro-enteritis, and bowel carcinoma need to be excluded. In addition, as it is so common, IBS can often co-exist with other disorders, a fact that needs to be recognised by physicians. In 1989, internationally agreed diagnostic criteria for IBS were drawn up by a group of renowned experts. These are known as the Rome I criteria and are frequently used by clinicians and researchers world-wide (Thompson W.G., Dotevall G., Drossman D.A. et al., 1989, IBS: guidelines for the diagnosis. Gastroenterology 2: 92-95). They are essentially the same as those proposed by Manning et al

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(Manning A.P., Thompson W.G., Heaton K.W. & Morris A.F., 1978, Towards positive diagnosis of the irritable bowel. Br. Med. J. 2, 653-4). See also Camilleri and Prather, 1992, The irritable bowel syndrome: mechanisms and a practical approach to management. Ann. Intern. Med. 116, 1001-8).

In summary, the criteria are as follows:

Clinical features

A. Continuous or recurrent side effects for 3 months or more, viz.:

10 1. Abdominal pain, relieved with defecation, or associated with a change in frequency or consistency of stool, AND/OR

B. Irregular or varying side effects 25% or more of the time including 3 or more of the following:

- 15 1. Altered stool frequency
 2. Altered stool consistency
 3. Altered stool passage (straining or urgency, feeling of incomplete evacuation)
 4. Passage of mucus
20 5. Bloating or feeling of abdominal distension.

Effective amount.

The effective amount of the compound or its salt to be administered will ultimately be at the discretion of the physician, taking into account the severity of the disease in
25 a particular subject (e.g. a human patient or animal model) and the overall condition of the subject. Suitable dose ranges will typically be in the range of from 0.01 to 20 mg/kg/day, preferably from 0.1 to 10 mg/kg/day.

2-Amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine.

30 This compound may be manufactured by reference to WO97/44326,

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the contents of which are incorporated herein by reference.

A salt or N-oxide of 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine.

5 A salt of this compound may be an acid addition salt in which the base retains the biological effectiveness and properties of the compound and which is physiologically acceptable. Such salts include those formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as
10 acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid
15 and the like.

"N-oxide" refers to the stable amine oxide formed at one of the pyrimidine nitrogen atoms.

Acid addition salts and N-oxides may be produced by means conventional in the art.

20 *Treatment or therapy.*

These terms refer to any administration of the compound, salt or N-oxide thereof, intended to alleviate the severity of a disorder of the GI tract in a subject, and includes treatment intended to cure the disease, provide relief from the symptoms
25 of the disease and to prevent or arrest the development of the disease in an individual at risk from developing the disease or an individual having symptoms indicating the development of the disease in that individual.

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Compositions and their administration.

Compositions may be formulated for any suitable route and means of administration. Pharmaceutically acceptable carriers or diluents include those used in formulations suitable for
5 oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of
10 the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association
15 the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

For solid compositions, conventional non-toxic solid carriers include, for example, pharmaceutical grades of mannitol,
20 lactose, cellulose, cellulose derivatives, starch, magnesium stearate, sodium saccharin, talcum, glucose, sucrose, magnesium carbonate, and the like may be used. The active compound as defined above may be formulated as suppositories using, for example, polyalkylene glycols, acetylated
25 triglycerides and the like, as the carrier. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc, an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline aqueous dextrose,
30 glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH

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buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 15th Edition, 1975. The composition or formulation to be administered will, in any event, contain a quantity of the active compound(s) in an amount effective to alleviate the symptoms of the subject being treated.

Dosage forms or compositions containing active ingredient in the range of 0.25 to 95% with the balance made up from non-toxic carrier may be prepared.

For oral administration, a pharmaceutically acceptable non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example, pharmaceutical grades of mannitol, lactose, cellulose, cellulose derivatives, sodium crosscarmellose, starch, magnesium stearate, sodium saccharin, talcum, glucose, sucrose, magnesium, carbonate, and the like. Such compositions take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like. Such compositions may contain 1%-95% active ingredient, more preferably 2-50%, most preferably 5-8%.

Parenteral administration is generally characterized by injection, either subcutaneously, intramuscularly or intravenously. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like. In addition, if desired, the pharmaceutical

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compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate,
5 triethanolamine oleate, triethanolamine sodium acetate, etc.

A more recently devised approach for parenteral administration employs the implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained. See, e.g., US Patent No. 3,710,795.

10 The percentage of active compound contained in such parental compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject. However, percentages of active ingredient of 0.1% to 10% in solution are employable, and will be higher if
15 the composition is a solid which will be subsequently diluted to the above percentages. Preferably, the composition will comprise 0.2-2% of the active agent in solution.

The following example illustrates the invention.

Materials and Methods

20 Strips of human colon longitudinal muscle were prepared as follows:

A section of human colon was cut open along its longitudinal axis. The section was pinned out flat and the mucosa carefully removed using sharp dissecting scissors. Once the
25 mucosa was removed, the section was turned over to reveal the three taenia coli (taenia mesocolica, taenia omentalis and taenia libera) and the muscle bands that lie between them. Longitudinal muscle strips (2mm wide by 20mm long) were then cut from the tissue between the taenia coli and suspended

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between stainless steel hooks in organ chambers containing oxygenated (95% O₂/5% CO₂) Krebs solution at 37°C. The composition of the Krebs solution was as follows: NaCl (118.2mM), KCl (4.69mM), MgSO₄·7H₂O (1.18mM), KH₂PO₄ (1.19mM),
5 glucose (11.1mM), NaHCO₃ (25.0mM), CaCl₂·6H₂O (2.5mM).

Tissues were placed under a tension equivalent to 10mN and left to equilibrate for a period of at least 60 minutes. Responses were recorded using isometric transducers coupled to an Apple Macintosh computer via a MacLab interface. After 60
10 minutes, the longitudinal muscle sections of the human colon were stimulated electrically (sub-maximal voltage and frequency with 60s between successive stimulations), using parallel platinum wire electrodes and a Multistim D330 pulse stimulator. Upon electrical stimulation, the strips of human
15 colon longitudinal smooth muscle responded with a rapid contraction.

Once the response to electrical stimulation had stabilised (successive stimulated responses differed by no more than 10%), the strips of human colon longitudinal smooth muscle
20 were exposed to increasing concentrations of 5-HT or 5-HT receptor agonist, or 5-HT in the presence of 5-HT receptor antagonist.

Potent enhancement of electrically-induced contractions of longitudinal muscle strips from human colon by low
25 concentrations of 5-HT (about 10⁻⁹ to 10⁻⁷M) was observed.

Reference Example 1 - Effects of 5-HT receptor agonists.

Application of 5-HT, as well as various 5-HT receptor agonists, has been shown to produce a potent enhancement of the contractile response to electrical stimulation. The
30 electrically-induced contractile response is inhibited by both

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tetrodotoxin and atropine, indicating that the effect is neuronal in nature, and is mediated via the release of acetylcholine.

5 The order of agonist potency at enhancing the contractile response in colon longitudinal muscle was determined according to the method outlined above. The results are shown in Table 1, and compared to the mean pEC_{50} for agonists at the 5-HT receptor in colon (mean \pm s.e.mean for at least 3 donors) and also at human 5-HT_{2A}, 2B, 2C and 5-HT₄ receptors whereby all data
10 are for human 5-HT receptors. NA indicates that data are not available at the human receptor.

Table 1

Agonist	Colon	5-HT _{2A}	5-HT _{2B}	5-HT _{2C}	5-HT ₄
Alpha-Me-5-HT	8.5 \pm 0.1	NA	8.4	NA	6.2
15 5-HT	8.2 \pm 0.1	7.4	8.1	8.5	6.7
2-Me-5-HT	7.0 \pm 0.4	NA	6.7	NA	6.0
5-MeOT	6.8 \pm 0.2	NA	7.6	NA	6.2
Cisapride	5.8 \pm 0.3	NA	NA	NA	5.5

20 Table 1. Potencies of some selective receptor agonists at the 5-HT receptor in human colon longitudinal muscle.

The order of agonist potency implicates a receptor of the 5-HT₂ family.

Reference Example 2 - Effects of 5-HT receptor antagonists.

25 The effects of various selective 5-HT receptor antagonists against 5-HT induced activity was also tested, according to the methods set out above. The affinities of selective receptor antagonists at the 5-HT receptor in human colon longitudinal muscle was determined (for at least 3 donors) and

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compared to the mean pK_b or pA_2 for antagonism at human 5-HT_{2A},
 2B, 2C and 5-HT₄ receptors. The results are shown in Table 2.
 Responses to 5-HT was measured in human isolated tissues whose
 5-HT receptor characteristics have been described previously
 (*) or from binding data at human recombinant receptors (#).
 NA indicates data are not available at the human receptor, NSA
 indicates non-surmountable antagonism. The profile generated
 corresponds to a receptor of the 5-HT_{2B} receptor class.

Table 2

Antagonist	Colon	5-HT _{2A}	5-HT _{2B}	5-HT _{2C}	5-HT ₄
SB 206553	8.5±0.1	6.0 [#]	9.0 [*]	7.9-9.0 [#]	NA
Rauwolscine	7.9±0.2	6.6 [#]	7.0-7.8 [#]	5.8 [#]	NA
Yohimbine	7.5±0.2	5.3 [#]	8.0 [*]	<5.0 [#]	NA
Methiothepin	7.5±0.3	NA	8.1 [#]	NA	<6.0 [*]
Cisapride	7.1±0.1	8.0 [#]	7.2 [#]	6.3 [#]	NA
SB 204741	6.8±0.2	5.2 [#]	6.7 [#]	5.8 [#]	<5.0
SB 242084	6.3±0.2	6.8 [#]	7.0 [#]	<6.0 [#]	<5.0 [#]
Ketanserin	6.1±0.2	8.6 [*]	<6.0 [*]	6.8 [*]	4.7 [*]
Methysergide	NSA at -9M	8.4 [#]	9.6 [*]	8.9 [*]	<5.0 [*]

Table 2. Affinities of some selective receptor antagonists at
 the 5-HT receptor in human colon longitudinal muscle and
 comparisons.

Example - Effect of RS127445 on colon longitudinal muscle.

Concentrations between 0.3nM and 100nM of RS127445 were found
 to antagonise the effects of 5-HT in electrically-stimulated
 longitudinal muscle strips of human colon (experiments
 performed as described above) with a pK_b of 9.5 (see Table 3).
 The antagonism produced by RS127445 is shown in Figure 1,
 which shows the response to 5-HT in the absence (control, ■)
 and presence of RS127445 at a concentration of 1nM (●) and

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100nM (○). All data are expressed as a percentage of the maximum response to 5-HT, and are given as mean \pm s.e. mean for $n > 4$ donors. Schild analysis of this antagonism yielded a plot with slope of 0.93 ± 0.17 , in accordance with competitive antagonism. Thus RS127445 is a potent ($pK_B = 9.45 \pm 0.41$) and selective antagonist of the effects of 5-HT at the 5-HT_{2B} receptor in human colon, showing that this compound may be used in the treatment of IBS and other GI disorders.

Table 3

Antagonist	Colon	5-HT _{2A}	5-HT _{2B}	5-HT _{2C}	5-HT ₄
RS 127445	9.45 ± 0.41	6.3 ± 0.2	9.5 ± 0.1	6.4 ± 0.1	NA

Summary of Examples.

In summary, the functional ligand profile at the receptor that mediates 5-HT-induced potentiation of the neuronally-mediated contractile response in human colon corresponds to that at the 5-HT_{2B} receptor sub-type. This indicates therefore that a selective antagonist at this receptor is able to counteract the hypermotility effects of 5-HT in the human intestine, and thereby represents an effective treatment for IBS. RS127445 (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine) is both more potent than other antagonists on colon longitudinal muscle, and more selective for the 2B receptor subtype compared to the 2A and 2C receptor subtypes than other antagonists. Thus the use of RS127445 in the treatment of GI disorders, in particular IBS, is indicated by the present novel findings.

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CLAIMS

1. A method for the treatment of a disorder of the GI tract, which method comprises administering to a subject in need of treatment, an effective amount of 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine, or a salt or N-oxide thereof.
2. The method of claim 1 wherein said salt is the hydrochloride or maleate salt.
3. The method of claim 1 wherein said compound is in the form of a composition comprising said compound and a pharmaceutically acceptable diluent or carrier.
4. The method of claim 1 wherein said disorder is IBS.
5. The use of 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine, or a salt or N-oxide thereof, in the manufacture of a medicament for the treatment of a disorder of the GI tract.
6. Use according to claim 5 wherein said salt is the hydrochloride or maleate salt.
7. Use according to claim 5 or 6 wherein said compound is in the form of a composition comprising said compound together with a pharmaceutically acceptable diluent or carrier.
8. Use according to any one of claims 5 to 7 wherein said disorder is IBS.

Figure 1

